Development of efficient synthetic promoters derived from plant pararetroviruses

A synopsis of the proposed work for

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Submitted by

Dipinte Gupta

Dr. Rajiv Ranjan
Supervisor

Prof. J N Srivatava
Head, Department of Botany

Prof. Ravindra Kumar
Dean, Faculty of Science

Department of Botany
Dayalbagh Education Institute
(Deemed University)
Dayalbagh, Agra
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INTRODUCTION

Promoters are specific sequence of nucleotides comprises of TATA box which serve as the start site of transcription (Dynan and Tjian, 1985). It can be studied by dividing into two sections, proximal region (core promoter) and distal region (*cis*-acting elements). Core promoter dictates the location of transcription to RNA Polymerase II, whereas cis-acting elements define the strength of promoter in terms of its expressivity, indirectly capability of forming proteins (Nikolov *et al.*, 1996; Berk 1999). *Cis*-acting element can be found hundreds or thousands of base pairs away from the gene location, upstream of coding region, within the coding or intronic sequence or even they can be located adjoining the promoter (Novina and Roy 1996). The arrangement of upstream *cis*-elements followed by a minimal promoter sets the polarity of the promoter. Although minimal promoter is essentially yet it alone have weaker transcriptional activity, thereby cis-elements, to which tissue, development-specific transcription factors bind, individually or in combinations, usually determine the spatio-temporal expression pattern of a promoter at the transcriptional level (Benfey and Chua, 1990).

Promoter imparts qualitative and quantitative effect on transcription; hence manipulations in promoters can be done to achieve basic research to crop improvement to plant molecular pharming (Potenza *et al.*, 2004). The architecture of the promoter elements can be redesigned for modifying the gene expression. The elementary logic behind developing such modified promoters is to transfer an upstream DNA sequence/*cis*-element that binds a specific *trans*-factor from one promoter into a different promoter containing the TATA sequence which might result in a novel regulatory or transcription model (Edelman *et al*., 2000).

The expression of a gene can be regulated at different stages of its expression among which transcription holds an importance. The promoters that derive this transgene expression ensure this control (Buchanan *et al.*, 2000). This technique had been exploited for exploration

and manipulation of biochemical processes, for the production of value added crops, for the production of nutraceuticals and pharmaceuticals to improve the human health, for phytoremediation and production of biodegradable plastics, antigens, antibodies toxins and many more (Sharma and Sharma 2009, Spok, 2007; Fischer *et al*., 2004; Twyman *et al*., 2003; Stoger *et al*., 2002).

1.1 Types of Promoter

On the basis of functionality promoter can be further classified as constitutive promoter, tissue-specific or development-stage-specific promoters, inducible promoter and synthetic or artificial promoter. Constitutive promoters induce a throughput expression of gene usually active across the species irrespective of environmental or development factor (Wang and Oard 2003, McElroy *et al.*, 1990, Huang *et al*., 2006). Tissue-specific or development-stage-specific promoters usually control the gene expression in tissue or developmental stage-specific manner. They are useful to accumulate the transgenic product in certain organs (Sunilkumar *et al*., 2002, Kluth *et al*., 2002). Inducible promoter persuades the transcriptional efficiency of the promoter either by chemicals such as tetracycline, alcohol, steroids, or physically by environmental factors like as desiccation, salt stress, temperature, light etc (Cramer and Weissenborn, 1997). Synthetic promoters are the artificially constructed by fusing a basal or core promoter with composite, *cis*-acting elements that have the ability to function independently from corresponding native promoter (Wang *et al*., 2003; Gurr and Rushton 2005; Tavva *et al*., 2006; Venter and Botha, 2010).

1.2 Native Promoters:

Promoters found in natural conditions in genome for the expression of gene, on which no genetic engineering had been done are known as native promoters. As every gene is regulated

by its individual promoters, hence there are endless examples of native Promoter. Several transcriptional promoters have been characterized from virus genome. A full-length transcript promoter and sub genomic transcript promoter are two major types of transcriptional promoter present in Caulimovirus genome. *Cis* acting element of retro elements of several viral promoters has played a significant role in promoter engineering by contributing its *cis* acting element of promoter (Singh *et al.*, 2002). In dicots the strongest promoter in use are predominantly from viral origin. Promoters from plant housekeeping genes like actin or ubiquitin have been used primarily in monocots. However, there is a risk of multiple transformations by a single promoter is that it might lead to silencing of both genes in successive generation through homologous recombination (Bhullar et al., 2003). In nature, this may be the one of the reasons why every gene in an organism is expressed from an individual promoter that has different DNA sequence. Recently, two promoters AtBANpro and $AtFUL_{pro}$ of genes BANYULS and FRUITFULL respectively of arabidopsis has shown enhanced expression in comparison to CaMV35S (Borghi and Xie 2016).

1.3 Synthetic Promoters:

To deal with the problem arises from native promoters in term of expressivity, Homology Based Gene Silencing (HBGS), inducibility etc. Worldwide scientist are working on. development of synthetic promoters by using several strategies which includes hybridization (Ranjan *et al*., 2012), DNA shuffling (Maiti *et al*., 1997), Domain Swapping (Bhullar *et al*., 2003), Intron mediated (Callis *et al*., 1987). A strong pathogen inducible promoter was made by hybridizing E17 and *cis*-acting elements upstream of CaMV 35S minimal promoter which are named as SPEE, SP-FF and SP-FFEE, (Shokouhifar *et al*., 2011). Similarly 4D and 2S2D are two efficient promoter constructed by fusing with p50 helicase domain of TMV replicase to meet

the objective of crown gall disease. Several abiotic stress inducible synthetic promoters had been developed which includes P1301A, P1301B (Chaturvedi *et al*., 2006), Pb12×ABRE, Pb14×ABRE (Ganguly *et al*., 2001) are salinity inducible promoter and NRP (Wang *et al.,* 2010) is an nitrate inducilbile promoter, whereas EFCFS-HS-1, EFCFS-HS-2,EFCFSHS-3 (Ranjan *et a.,l* 2012) are vascular tissue specific stress inducible promoter. A novel synthetic 35S promoter was developed using the approach of domain swapping and by replacing *cis*-element elements with *cis*-element having no intervening homologous sequences (Bhullar *et al*., 2003). MSgt-FSgt is an hybrid promoter formed by ligation of MMV and FMV promoter fragments has showed maximum GUS activity in tobacco and arabidopsis plants (Kumar *et al*.,2011) FUAS35SCP and MUAS35SCP are the two other synthetic promoters formed by intermolecular hybridization had shown four to five times stronger activity as compared to CaMV (Patro *et al.,* 2012). Several synthetic promoters had been developed that can be listed as 4XW1, 4XD, 4XGCC, 4XJERE, induced by oomycete-derived peptide and they regulate gene expression in local wounds (Rushton *et al*., 2002). E17-27 is induced by flagellin peptide elicitor found to have two fold higher GUS activity compared to monomer in arabidopsis (Kirsch *et al*., 2001).

1.4: Future Prospective:

With the economic point of view the fuel for driving any industry is the ability to synthesize complex molecules in a cost effective manner, least safety issue i.e. pathogen contamination and easy scale up (Lal *et al*., 2007; Rybicki, 2009). Plant molecular pharming is the field of science in which plants are engineered for the production of recombinant pharmaceutical and industrial proteins in large quantities in a cost effective manner. Hereby developing strong promoter as the deliverable expect of the present study one can over express a ectopic gene which ultimately increases the production of protein, hence we can meet the market demand. There are several examples of promoter used in plant molecular pharming which includes E8 promoter of tomato used for expression of various antigens in fruits Deikman *et al.,* 1992. rbcS promoter used for production of smallpox subunit vaccine (Dai *et al.,*2000; Golovkin *et al.,* 2007; Hyunjong *et al.,* 2006). CTB, LTB protective antigen or insulin have have been produced in chloroplasts using either Prrn or psbA promoter (Daniell *et al.,* 2001a, Kang *et al.,* 2004, Ruhlman *et al.,* 2007). Apart from plant molecular pharming synthetic promoters have a wide scope in development of abiotic and biotic stress tolerance plants, in neutraceuticals, in phytoremediation and degradation of Xenobiotic (Zanella *et al*., 2016).

For the proposed piece of work an effort would be taken to overcome the problems of the existing promoter by synthesizing a hybrid heterogeneous promoter utilizing promoters fragment of viral origin which infects monocot and dicots plants. Heterogeneity in promoter derived from pararetrovirus will solve the risk of HBGS and make it suitable for its universal use.

Aims & Objectives:

1. Isolation of promoter fragments from plants *pararetrovirus*.
2. Generation of synthetic promoter by domain swapping and mixing and exchanging of promoter fragments.
3. Evaluation of such synthetic promoters in monocot and dicot plant system.

REVIEW OF LITERATURE:

Since 1980 researchers are working on development of a competent promoter for gene transfer technologies. CaMV 35S most commonly used promoter was isolated by Chua and his colleagues at Rockefeller University. CaMV 35S promoter is responsible for the transcription of whole genome of *Cauliflower Mosaic Virus* and was named as CaMV 35S due to coefficient of sedimentation of the viral transcript (Benfey & Chua 1990). Minimal promoter of CaMV 35S contains the TATA box is present between -90 position to +1 transcription start site. Along with TATA box minimal promoter of CaMV 35S also contains CAAT-like boxes, these sequences has the potential to influence the efficiency of promoter. Enhancer region of CaMV 35S is located between -343 to -90 positions. This promoter is a strong constitutive promoter and causes high level of gene expression in dicot plants but less effective in monocot plants (McElroy et al., 1990).

Mirablis Mosaic Virus (MMV) belongs to family *Caulimovirus* (Richins and Shepherd 1983), and it infects Mirabilis plant species which are generally found in warmer parts of North America. MMV full length promoter and Caulimovirus contains several similar putative regulatory domains such as TATA box sequence TATATAA, CAAT sequence 63 bp upstream of the TATA box, poly (A) signal possessing AATAAA sequence located 204 bp downstream of the TATA box. Several repetitive sequence are found in MMV which are not found in Caulimovirus may possess an essential role in regulatory function of this virus. These repetitive sequence are located upstream of TATA box and direct repeat of 18 bp and 10 bp are found 3`of the TATA box (Lam 1994).

Rice Tungro Bacilliform Virus (RTBV) is a member of plant pararetrovirus group having double stranded DNA genome 8 kb (Hay *et al*., 1991). Promoter from this virus proved to be functional in transgenic rice plants, although its expression is limited in phloem which provides it's an tissue specificity (Yin and Beachy, 1995). RTBV found to have promoter that contains Box I, BoxII and two other *cis* elements for its proper functioning, they found that these *cis*-acting elements act synergistically and add phloem specific expression. Another group of Mathur and Dasgupta had worked with this RTBV promoter by carrying out an upstream and downstream deletion analysis (Mathur and Dasgupta 2007). They had conducted out and eleven deletion derivatives of full length promoter which comprises of five upstream deletion and six downstream deletions. They had found that -36 to +645 region gives expression equivalent to full-length promoter and deletion of down streaming (–231 to +58) (–231 to +23), region converts this tissue specific promoter into constitutive for the entire life of plant. These conclusions can be used for the redesigning the promoter architecture and development of novel promoters

In 1990 McElory and his collaborators had isolated an actin promoter for use in rice transformation. In a study conducted by actin minimal promoter was fused with promoter fragment of wheat GstA1 and potato Gst1 having GUS as an reporter gene. The result had shown that Act1 gene intron 1 of rice Act1 gene is important for heterogenic expression of monocot and dicot promoter element in rice. Although rice Act 1 promoter had shown enhanced constitutive expression in monocot plants, it had failed shown good expression pattern in dicot plants. Now days this actin promoter is combination with *cis*–element are synthesizing in scope of developing a strong chimeric promoter.

Ubiquitin is another strong constitutive promoter of monocots used in various studies and had shown an enhanced expression of gene. Wang had isolated the rice ubiquitin promoter RUBQ1 and RUBQ2 and checked its inducibility pattern (Wang et al., 2003). GUS expression profile had shown 8 to 35 fold higher expression with RUBQ and RUB2 respectively in transgenic rice. They had also conducted Deletion analysis of the 5` upstream region of RUBQ2 which had shown that a putative enhancer region of 74 bp sequences are presenting between 739 to 666 bases upstream of TATA box deletion of which causes 2.4 fold reduction in the activity of RUBQ2 promoter. Sivamani and Qu found that by fusing a monomer of nine neucleotide along with the core promoter of rice ubuiquitin gene rubi3 expression of transgenic can be enhanced by 4.3 fold, They had also found mutagenesis in the third neucleotide of this monomer still maintains the enhancing effect in reporter gene GUS but does not translate fusion protein (Sivamani & Qu., 2006).

Similarly Tao and his collaborators (Tao *et al*., 2015) characterized ubiquitin promoter of dicot plant jatropha curcas and compared its expression with CaMV35S. GUS-assay revealed that ubiquitin and CaMV 35S are equally expressed in stem, mature leaves and female flowers and jatropha ubiquitin promoter retains its activity in stress condition which shows that it can be a useful alternative of CaMV35S.

Several limitations had been faced with native or naturally occurring promoters in terms of their induction capability, their efficiency of transcription and their size. Hence strength and specificity of native promoter can be architecture by manipulating their *cis* elements using recombinant DNA technologies. Based on the interaction of *cis*-trans element present in a promoter, series of synthetic promoter have been developed which regulates varieties of

expression in plants including constitutive, stress inducible and tissue specific (Acharya *et al*., 2014; Bestwick and Kellogg 2000; Chaturvedi *et al* ., 2006).

Using the approach of Hybridization and DNA shuffling Ranjan et al., 2012 had developed synthetic promoters. They had used Figwort Mosaic Virus full-length transcript promoter (F) and sub-genomic transcript promoter (FS). Total six promoters was synthesized by this group two with single shuffling two with double shuffling and two hybrid promoters. On comparing the expression of synthetic promoter with CaMV 35S they found that hybrid promoter had shown enhanced expression while shuffled promoter had shown reduced activities. So they had also calculated free energy of hybrid promoter and shuffled promoter. Free energy had an essential role in DNA unwinding and facilitating RNA polymerase and other transcription factors during transcription process. Calculated free energy was much more less in shuffled promoter than hybrid promoter which might be a possible reason for better expression of hybrid promoters. This piece of study leads to a conclusion that hybridization is a better approach for development of synthetic promoter.

Recently Wang and his collaborators had identified green tissue specific promoters and synthesized strong promoters by reshuffling the architecture of cis-elements present by using bioinformatics tools and microarray technology (Wang *et al.,* 2015). Similarly novel *cis*-acting elements had identified in GmERF3 promoter which shows enhanced expression in transgenic plants, there is an differential expression pattern of this promoter in different tissues and in different plants. A synthetic promoter was developed by Garcia & Finer *et al.,* 2016 by identifying the *cis*-elements of GmERF3 promoter using bioinformatics tools and validating the results by forming synthetic promoters. Their synthetic promoter had shown increased expression, but 1-2 days after mechanical wounding thus they named it as delayed expression

elements. Scientist are also using promoters for enhanced of genes responsible for phytoremediation such as CaMV 35S-TMV omega promoter used for the overexpression of ADII gene which is responsible degradation of naphthalene. Hence Plants can be raised having an enhanced degradation efficiency of naphthalene hence reduced cancer risk (Xiao *et al*., 2016).

By reviewing the literature, it is concluded that many promoters have been discovered yet a competent promoter has to be discovered that can expressed in both monocot and dicot plants . As earlier reported repetitive use of a single promoter for gene expression, thus leads to a homologous based gene silencing (HBGS), thus alternative strategies can be employed to avoid HBGS. Although several promoter are available in literature some of them are highly efficient for expression in monocots and some in dicots still there are paucity of promoters suitable for various plant system.

Proposed Methodology

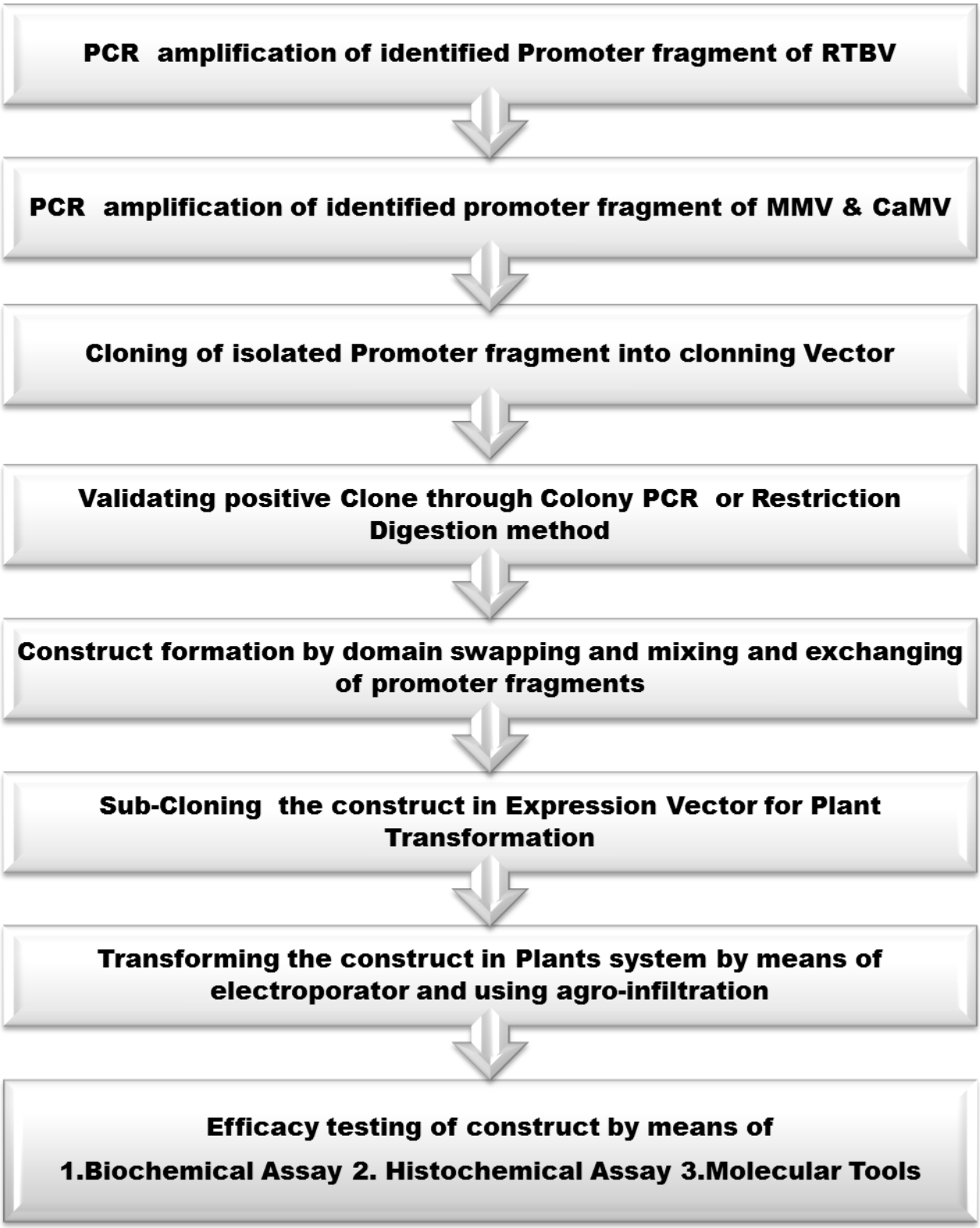
- Promoter fragments: Promoter of three viruses Mirablis *Mosaic Virus, Cauliflower Mosaic Virus, Rice Tungro Bacillus Virus* will be opted for this study.
- Reporter Gene: For the present study reporter gene GUS will be used for detecting the expression of construct.
- Model Plants: For testing the efficacy of the synthetic promoter a dicot plant tobacco and monocot plant rice will be selected.
- Cloning Vector: Cloning of the promoter fragment will be done in pUC119.
- Expression Vector: For expressing the formed construct in plants two expression vector pUCPMAGUS and pKYLXGus will be chosen.
- Isolation of Promoter fragment: Cloning of a promoter fragment involved following basic steps comprises of designing of appropriate primers, PCR amplification, purification of amplified fragment, Restriction digestion of purified fragment and suitable vector, ligation of insert and vector, transformation of ligated product. Finally screening of transformed cells through colony PCR.
- Construct Formation: Promoter fragment from two different sources are hybridized and will be sub cloned in pUCPMAGUS and pKYLXGus vector to form an expression vector.
- Transformation in Plants: The formed construct will be transformed into plants by means of agro-infiltration and electroporator.
- Efficacy testing of construct: To analyze the efficacy level of the construct three modes are chosen which includes biochemical Assay, histochemical Assay and molecular Analysis.

- Biochemical Assay: The plants material will be incubated in MUG buffer and expression analysis will be conducted using luminometer (Jefferson *et al*., 1987).

- Hitochemical Assay: Thin Section from various parts of plants will be incubated with GUS-buffer at 37 C for 24 hours. The activity was compared with development of color (Ranjan *et al*., 2012).

- Molecular Analysis: Several molecular techniques are available for detection of gene expression. The technique which will be chosen for the present study includes RT (Reverse transcription) PCR (Ranjan *et al*., 2011).

Overview of Work Plan


PCR amplification of identified Promoter fragment of RTBV
PCR amplification of identified promoter fragment of MMV & CaMV
Cloning of isolated Promoter fragment into clonning Vector
Validating positive Clone through Colony PCR or Restriction Digestion method
Construct formation by domain swapping and mixing and exchanging of promoter fragments
Sub-Cloning the construct in Expression Vector for Plant Transformation
Transforming the construct in Plants system by means of electroporator and using agro-infiltration
Efficacy testing of construct by means of
1.Biochemical Assay 2. Histochemical Assay 3.Molecular Tools

References

1. Acharya S, Ranjan R, Pattanaik S, Maiti IB, Dey N (2014). Efficient chimeric plant promoters derived from plant infecting viral promoter sequences. Planta 239:381–396.
2. Benfey PN, Chua NH (1990). The Cauliflower Mosaic Virus 35S Promoter Combinatorial Regulation of Transcription in Plants. Science 250:959-966.
3. Berk AJ (1999). Activation of RNA polymerase II transcription. Current opinion in cell biology 11:330-335.
4. Bestwick RK, Kellogg JA (2000). Synthetic hybrid tomato E4/E8 plant promoter. US Patent 6,118,049, 2000.
5. Bhullar S, Chakravarthy S, Advani S, Datta S, Pental D, Burma PK (2003). Strategies for development of functionally equivalent promoters with minimum sequence homology for transgene expression in plants: *cis*-elements in a novel DNA context versus domain swapping. Plant physiology 132:988-998.
6. Bhullar S, Datta S, Burma PK (2010). Delayed trans-inactivation of synthetic domain A 35S promoters by ‘‘Tobacco 271 Locus’’ due to reduced sequence homology. Plant Mol Biol Rep 29:1–11.
7. Borghi M and Xie DY (2016). Tissue specific production of limonene in Camelinasativa with the Arabidopsis promoters of genes BANYULS and FRUITFULL. Planta 243(2): 549-561.
8. Buchanan BB Gruissem W, Jones R, (2000). Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists 340-342.
9. Callis J, Fromm M, Walbot V (1987). Introns increase gene expression in cultured maize cells. Genes & development 1:1183-1200.
10. Carlos M. Hernandez-Garcia CMH and Finer JJ (2016). A novel *cis*-acting element in the GmERF3 promoter contributes to inducible gene expression in soybean and tobacco after wounding. Plant Cell Rep 35:303–316.
11. Chaturvedi CP, Sawant SV, Kiran K, Mehrotra R, Lodhi N, Ansari S A &Tuli R (2006). Analysis of polarity in the expression from a multifactorial bidirectional promoter designed for high-level expression of transgenes in plants. J. Biotechnol. 123:1-12.
12. Cramer CL, Weissenborn DL, Oishi KK, Grabau EA, Bennett S, Ponce E (1996). Bioproduction of human enzymes in transgenic tobacco. Ann N Y Acad Sci 792:62–71.

13. Dai Z, Hooker BS, Anderson DB, Thomas SR (2000). Expression of Acido thermos cellulolytic usendo glucanase E1 in transgenic tobacco biochemical characteristics and physiological effects. Transgenic research 9:43-54.
14. Daniell H, Lee SB, Panchal T, Wiebe PO (2001). Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts. Journal of molecular biology 311:1001-1009.
15. Deikman J, Fischer RL (1988). Interaction of a DNA binding factor with the 5'-flanking region of an ethylene-responsive fruit ripening gene from tomato. The EMBO journal 7:3315-3320.
16. Dynan, WS and Tjian R (1985). Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. Nature 316:774-778.
17. Edelman GM, Meech R, Owens GC, Jones FS (2000). Synthetic promoter elements obtained by nucleotide sequence variation and selection for activity. Proceedings of the National Academy of Sciences of the United States of America 97:3038-3043.
18. Featherstone M (2002) Coactivators in transcription initiation: here are your orders. Current opinion in genetics & development 12:149-155.
19. Fischer R, Stoger E, Schillberg S, Christou P, Twyman RM (2004). Plant-based production of biopharmaceuticals. CurrOpin Plant Biol 7:152–8.
20. Ganguly M, Roychoudhury A, Sarkar SN, Sengupta DN, Datta SK &Datta K (2011) Inducibility of three salinity/abscisic acid-regulated promoters in transgenic rice with gusA reporter gene. Plant Cell Rep 30:16177-162.
21. Golovkin M, Spitsin S, Andrianov V, Smirnov Y, Xiao Y, Pogrebnyak N, Markley K, Brodzik, R., Gleba Y, Isaacs SN, Koprowski H (2007) Smallpox subunit vaccine produced in Planta confers protection in mice. Proceedings of the National Academy of Sciences of the United States of America 104:6864-6869.
22. Gurr S, Rushton PJ (2005) Engineering plants with increased disease resistance: how are we going to express it? Trends in biotechnology 23:283-290.
23. Hay, J. M., M. C. Jones, M. L. Blakebrough, I. Dasgupta, J. W. Davies, and R. Hull (1991). An analysis of the sequence of an infectious clone of rice tungro bacilliform virus, a plant pararetrovirus. Nucleic Acids Res. 19:2615– 2621.

24. Herley CB and Reynold RP (1987). Analysis of E. coli promoter. Nucleic Acid Res 15:2343-2361
25. Huang Z, Santi L, LePore K, Kilbourne J, Arntzen CJ, Mason HS (2006). Rapid, high-level production of hepatitis B core antigen in plant leaf and its immunogenicity in mice. Vaccine 24:2506-2513.
26. Hyunjong B, Lee DS, Hwang I (2006). Dual targeting of xylanase to chloroplasts and peroxisomes as a means to increase protein accumulation in plant cells. Journal of experimental botany 57:161-169.
27. Jefferson RA, Kavanagh TA, Bevan MW (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. The EMBO journal 6:3901-3907.
28. Kang TJ, Kang KH, Kim JA, Kwon TH, Jang YS, Yang MS, (2004). High-level expression of the neutralizing epitope of porcine epidemic diarrhea virus by a tobacco mosaic virus-based vector. Protein expression and purification 38:129-135.
29. Kirsch C, Logemann E, Lippok B, Schmelzer E, Hahlbrock K (2001). A highly specific pathogen-responsive promoter element from the immediate-early activated CMPG1 gene in Petroselinum crispum. Plant J Cell Mol Biol 26:217–227.
30. Kluth A, Sprunck S, Becker D, Lorz H, Lutticke S (2002). 5' deletion of a gbss1 promoter region from wheat leads to changes in tissue and developmental specificities. Plant molecular biology 49:669-682
31. Kumar D, Patro S Ranjan R Sahoo DK, Maiti IB, et al. (2011) Developmentof useful recombinant promoter and its expression analysis in different plant cells using Confocal Laser Scanning Microscopy. PLoS ONE 6(9): e24627.
32. Lal P, Ramachandran VG, Goyal R, Sharma R(2007) . Edible vaccines: current status and future. Indian J Med Microbiol 25:93-102.
33. Lam E. & Chua NH (1991) Tetramer of a 21-base pair synthetic element confers seed expression and transcriptional enhancement in response to water stress and abscisic acid. J. Biol. Chem. 266:17131- 17135.
34. Maiti IB, Gowda S, Kiernan J, Ghosh SK, Shepherd RJ (1997) Promoter/leader deletion analysis and plant expression vectors with the figwort mosaic virus (FMV) full length

transcript (FLt) promoter containing single or double enhancer domains. Transgenic research 6:143-156.

35. Mathur S and Dasgupta I (2007) Downstream promoter sequence of an Indian isolate of Rice tungro bacilliform virus alters tissue-specific expression in host rice and acts differentially in heterologous system Plant MolBiol 65:259–275.
36. McElroy D, Zhang W, Cao J, Wu R (1990). Isolation of an efficient actin promoter for use in Mascarenhas D, Mettler IJ, Pierce DA, Lowe HW Intron-mediated enhancement of heterologous gene expression in maize. Plant molecular biology 15:913-920.
37. Nikolov DB, Burley SK (1997). RNA polymerase II transcription initiation: a structural view. Proceedings of the National Academy of Sciences of the United States of America 94:15-22.
38. Nikolov DB, Chen H, Halay ED, Hoffman A, Roeder RG, Burley SK (1996). Crystal structure of a human TATA box-binding protein/TATA element complex. Proceedings of the National Academy of Sciences of the United States of America 93:4862-4867.
39. Novina CD, Roy AL (1996). Core promoters and transcriptional control. Trends in genetics: TIG 12:351-355.
40. Patro S, Kumar D, Ranjan R, Maiti IB, Dey N (2012). The development of efficient plant promoters for transgene expression employing plant virus promoters. Molecular plant 5:941-944.
41. Petruccelli S, Dai S, Carcamo R, Yin Y, Chen S, Beachy RN (2001). Transcription factor RF2a alters expression of the rice tungro bacilliform virus promoter in transgenic tobacco plants. PNAS 98(13):7635–7640.
42. Potenza C, Aleman L, Sengupta-Gopalan C (2004). Targeting transgene expression in research, agricultural, and environmental applications: Promoters used in plant transformation. In Vitro Cellular & Developmental Biology - Plant 40:1-22.
43. Ranjan R, Patro S, Kumari S, Kumar D, Dey N, Maiti IB (2011). Efficient chimeric promoters derived from full-length and sub-genomic transcript promoters of Figwort mosaic virus (FMV). Journal of biotechnology 152:58-62.
44. Ranjan R, Dey N (2012). Development of vascular tissue and stress inducible hybrid-synthetic promoters through dof-1 motifs rearrangement. Cell biochemistry and biophysics 63:235-245.

45. Richins RD, Shepherd RJ (1983) Physical maps of the genomes of dahlia mosaic virus and mirabilis mosaic virus-two members of the caulimovirus group. Virology 124:208-214
46. Ruhlman T, Ahangari R, Devine A, Samsam M, Daniell H (2007). Expression of cholera toxin B-proinsulin fusion protein in lettuce and tobacco chloroplasts--oral administration protects against development of insulitis in non-obese diabetic mice. Plant biotechnology journal 5:495-510.
47. Rushton PJ, Reinstadler A, Lipka V, Lippok B, Somssich IE (2002). Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling. The Plant cell 14:749-762.
48. Rybicki EP (2009). Plant-produced vaccines: promise and reality. Drug Discov Today 14:16–24.sequence-specific DNA-binding proteins. *Nature*, 316 (6031): 774-778.
49. Sharma AK, Sharma MK (2009) Plants as bioreactors: Recent developments and emerging opportunities Biotechnology Advances 27 811–832.
50. Shokouhifar F, Zamani MR, Motallebi M, Mousavi A, Malboobi MA (2011) Construction and functional analysis of pathogen-inducible synthetic promoters in Brassica napus. Biol Plant 55:689–695.
51. Singh KB, Foley RC, Oñate-Sánchez L, (2002). Transcription factors in plant defense and stress responses. Current Opinion in Plant Biology 5:430-436.
52. Sivamani E and Qu R Expression enhancement of a rice polyubiquitin gene promoter (2006). Plant Molecular Biology 60:225–239.
53. Spok A (2006). From farming to "Pharming"; risks and policy challenges of third generation GMcrops. ITA-Manu:scripte;12:1-30.
54. Stoger E, Parker M, Christou P, Casey R (2001). Pea legumin overexpressed in wheat endosperm assembles into an ordered paracrystalline matrix. Plant Physiol;125:1732–42
55. Sunilkumar G, Connell JP, Smith CW, Reddy AS, Rathore KS (2002). Cotton alpha-globulin promoter: isolation and functional characterization in transgenic cotton, Arabidopsis, and tobacco. Transgenic research 11:347-359.
56. Tao YB, Liang LH, Long J N , Fu ZX (2015) Isolation and characterization of an ubiquitin extension protein gene (*JcUEP*) promoter from *Jatropha Curcas Planta* 241: 823836.

57. Tavva VS, Dinkins, RD, Palli SR, Collins GB (2006). Development of a methoxyfenozide-responsive gene switch for applications in plants. The Plant journal : for cell and molecular biology 45:457-469.
58. Twyman RM, Schillberg S, Fischer R (2005). Transgenic plants in the biopharmaceutical market.Expert OpinEmerg Drugs;10:185–218.
59. Venter M, Botha FC (2010). Synthetic Promoter Engineering, in: Pua, E.C., Davey, M.R. (Eds.), Plant Developmental Biology - Biotechnological Perspectives. Springer Berlin Heidelberg, pp. 393-414
60. Wang J, Oard JH (2003). Rice ubiquitin promoters: deletion analysis and potential usefulness in plant transformation systems. Plant Cell Report 129-34
61. Wang R, Guan P, Chen M, Xing X, Zhang Y, Crawford NM (2010). Multiple regulatory elements in the Arabidopsis NIA1 promoter act synergistically to form a nitrate enhancer. Plant Physiol 154(1) 423-432
62. Wang R, Zhu M , Ye R, Liu Z, Zhou F, Chen H &Lin Y (2015). Novel green tissue-specific synthetic promoter and *cis*-regulatory elements in rice. Scientific Report DOI 101038/strep18256.
63. Xiao YF, Zhu B, Han HJ, Tian YS, Pengu RH, Yao QH (2016) Enhancement of naphthalene tolerance in transgenic Arabidopsis plants overexpressing the ferredoxin-like protein (ADI1) from rice Plant Cell Rep 35:17–26
64. Yin Y and Beachy RN (1995). The regulatory region of the rice tungro bacilliform virus promoter and interacting nuclear factors in rice (Oryza sativa L.) The Plant rna 7(6):969-980.
65. Zanella LF, Patrizia B, Enrica R, Laura , Simone D A, Rovere FD, Cardarelli M, Barbieri5 M , Toppi LD, Degola S L, Altamura MM Giuseppina F (2016.) Overexpression of AtPCS1 in tobacco increases arsenic and arsenic plus cadmium accumulation and detoxification. Planta 243:605–622.